Gel Visualization Methods

Use fresh solutions/buffers for all steps (or solutions/buffers dedicated to proteomics' samples). Wear a clean lab coat, gloves, hat and face mask when handling the gel (especially when excising the bands/spots). Use cleaned glassware (2-3 washes with HPLC grade 50%ACN/water) and cleaned pipet tips (2-3 washes with the working solution/buffer).

Cover the gel container with food wrap as much as possible (i.e. do not expose the gel to air for too long). USE A GLASS CONTAINER.

Silver Stain:
LOD (visual) ~1-10 ng (not quantitative method)
USE OUR SILVER STAINING PROTOCOL, it's cheap and efficient (see below).
N.B. - Try to avoid over staining (bands that appear with over staining might not be identifiable by LC-MS/MS). Try to increase the amount of proteins instead.
- This distaining protocol causes artifacts (sulphonation/sulfation) for the identification of phosphorylated sites. Use Coomassie, colloidal Coomassie or fluorescent staining instead.

Coomassie Brilliant Blue (R-250):
Inexpensive, semi-quantitative, simple
LOD~ 60-100 ng.
Cover gel with staining solution and incubate from 0.5 to 4 hr/decant. (0.1% CBB R-250, 50% methanol, 10% acetic acid). Briefly rinse gel with analytical water to remove residual stain/decant.
Distain gel in 30-50% methanol: 5-10 % acetic acid: analytical water/decant
30 minutes soaking in water before excising the bands

Colloidal Coomassie (G-250):
LOD ~2-10 ng.
Wash gel 3 ´ (10-15 min) in analytical water to dilute SDS/decant.
Just cover gel with Colloidal Coomassie stain (~4-6 hr)/decant:
Rinse 2 ´ with analytical water (1 min)/decant.
Distain gel with analytical grade water until background is low (~4 hours).
30 minutes soaking in water before excising the bands

Fluorescent Methods:
Sypro Ruby/Red/Orange/Tangerine, Ruthenium II, Deep Purple
Recommended for both visualization and MS
Very sensitive, quantitative (orders of magnitude), simple, more expensive, required UV plate for visualization and band excision.
LOD ~1-10 ng, preferred method for low abundance proteins
Follow the manufacturer protocol.
*Caution: Photobleaching occurs with a “half life” of ~15 min in transilluminators.*
30 minutes soaking in water before excising the bands

**Silver staining for LC/MS/MS analysis**
Use a clean glass container to stain the gel and **do not touch the gel with your hands**. A large gel will require 250 to 500 ml of each solution. All washes and rinses should be performed while gently rocking the glass container.

1. **Fix the gel in 50% methanol: 10% acetic acid for 30 min. Repeat once at least 2h to O/N.**
   - 125 ml methanol + 25 ml acetic acid + 100 ml H₂O

2. **Rinse the gel with 20% ethanol for 20 min.**
   - 50 ml ethanol + 200 ml H₂O

3. **Rinse the gel in water for 20 min.**

4. **Reduce the gel with sodium thiosulfate (0.2 g/L) for 2 min.**
   - 0.05 g sodium thiosulfate + 250 ml H₂O
   
   **N.B. Keep 25 ml for step 8 (for the developing solution).**

5. **Rinse twice 20 sec with water.**

6. **Incubate in silver nitrate (2 g/L) for 30 min.**
   - 0.5 g silver nitrate + 250 ml H₂O

7. **Rinse once with water for 20 sec.**

8. **Wash the gel once with the developing solution (about 100-200 ml), discard the wash and develop to desired intensity with the remainder of the developing solution.**

   **Developing solution** contains sodium carbonate (30 g/L), formaldehyde (1.4 ml of a 37% solution/L), and sodium thiosulfate (10 mg/L, i.e. 25 ml of the previous solution, step 4, for 500 ml).
   - 15 g sodium carbonate + 475 ml H₂O
   - 25 ml of sodium thiosulfate (0.2 g/L) - step 4
   - 700 µl of formaldehyde
9. Stop the reaction by exchanging the developing solution with 1% (v/v) acetic acid. Incubate for a minimum of 30 min.

- 2,5 ml acetic acid + 250 ml H₂O

**IMPORTANT:** try to avoid developing the gel more than 4 minutes since over staining reduces sensitivity for proteomic analysis (developing time must be recorded).

Store at – 80°C, the bands can be stored frozen for months.

**Products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Code</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium thiosulfate</td>
<td>S446-500</td>
<td>Fisher</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td>S181-25</td>
<td>Fisher</td>
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<tr>
<td>Sodium carbonate</td>
<td>S263-3</td>
<td>Fisher</td>
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<tr>
<td>Formaldehyde</td>
<td>F79-4</td>
<td>Fisher</td>
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<tr>
<td>Acetic acid, glacial</td>
<td>A38 212</td>
<td>Fisher</td>
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